Reactivos GPL

Barcelona, España

CE

- ATHROM-III -

ANTITHROMBIN III
Turbidimetry

Store at: +2+8ºC.

Presentatión:
Cod. IT061 CONT: R1 1 x 40 mL. + R2 1 x 10 mL

Procedure

Diagnostic reagent for quantitative determination of human antithrombin III (ATHROM-III).

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY
The ATHROM-III is a quantitative turbidimetric test for the measurement of antithrombin III in human serum or plasma.
Anti-antithrombin III antibodies when mixed with samples containing antithrombin-III, form insoluble complexes. These complexes cause an absorbance change, dependent upon the antithrombin-III concentration of the patient sample, that can be quantified by comparison from a calibrator of know antithrombin-III concentration.

REAGENTS COMPOSITION

Diluent (R1)	Tris buffer 20 mmol/L, PEG 8000, pH 8.3. Sodium azide 0.95 g/L.
Antibody (R2)	Goat serum, antithrombin III, pH 7.5. Sodium azide 0.95 g/L.
Optional	General Protein Calibrator

REAGENT PREPARATION AND STABILITY
Antibody and diluent are ready to use.
All the components are stable up to the end of the indicated month and year of expiry. Stored at tightly closed at 2-*8ºC* and contaminations are prevented during their use.
Do not freeze; frozen reagents could change the functionality of the test.
Signs of reagent deterioration:
- Particles and turbidity indicates contamination or reagents deterioration.

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8ºC,. Do not use reagents over the expiration date.

CALIBRATION
It is recommended the use of the PROT CAL for calibrate the reagent. The reagent (both monoreagent and bireagent) should be recalibrated every month, when the controls are out of the specifications, and when changing the reagent lot or the instrument settings.

SPECIMEN
Fresh plasma. Sodium citrate should be used as anticoagulant. Stable 7 days at 2-8ºC or 3 months at –20ºC.
Do not use highly hemolized or lipemic samples.

Discard contaminated specimen.

MATERIAL REQUIRED BUT NOT PROVIDED
- Thermostatic bath at 37ºC.
- Spectrophotometer capable of accurate absorbance readings at 340 nm (320-360)
- Cuvettes with 1 cm light path.

General laboratory equipment

TEST PROCEDURE
CALIBRATION CURVE:
Prepare the following PORT-CAL dilutions in ClNa 9 g/L as diluent. Multiply the concentration of the ATROM-III calibrator by the corresponding factor stated in table bellow to obtain the ATROM-III concentration of each dilution.

Calibrator dilution	1	2	3	4	5	6
Calibrator (µL)	--	10	25	50	75	100
ClNa 9 g/L (µL)	100	90	75	50	25	-
Factor	0	0.1	0.25	0.5	0.75	1.0

MANUAL PROCEDURE
1. Bring the working reagent and the photometer (cuvette holder) to 37ºC.
2. Assay conditions:
   - Wavelength: ..... 340 nm.
   - Temperature: 37ºC
   - Cuvette light path: ..... 1 cm.
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

R_1: Diluent (µL)	800
Sample or Calibrator (µL)	20

5. Mix and read the absorbance (A_1) of the sample addition.
6. Immediately pipette into the cuvette:

R_2: Antibody (µL)	200

7. Mix and read the absorbance (A_2) of calibrators and samples exactly 5 minutes after the R_2 addition.

CALCULATIONS
Calculate the absorbance difference (A_2-A_1) of each point of the calibration curve and plot the values obtained against the ATROM-III concentration of each dilution. ATROM-III concentration in the sample is calculated by interpolation of its (A_2-A_1) in the calibration curve.

QUALITY CONTROL
General Protein Controls Ref.: IT220. is recommended to monitor the performance.

Serum Controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES
Between 17 – 30 mg/dL.

It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE
Antithrombin III is a protein synthesized in the liver, normally present in the human plasma. It is the major inhibitor of the thrombin, and inhibits coagulation and limits the forming of blood clots. Antithrombin-III is also capable of activating other components of the coagulation cascade (eg, factor Xa), as well as plasmin.
Antithrombin-III deficiency can cause or lead to thrombosis, a clot forming in a blood vessel. Clots forming in the legs and pulmonary embolism are most commonly reported. Antithrombin-III deficiency is usually inherited and affects males and females equally. All family members should be tested if there is history of the disease.
Acquired antithrombin-III deficiency can occur as a result of other conditions. It has been reported in patients with liver diseases, patients receiving certain kinds of chemotherapy, and patients using oral contraceptives.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE
- *Linearity:* Up to 70 mg/dL[note 1,2]. Under the described assay conditions.
- *Limit detection*: Values less than 7.4 mg/dL give non-reproducible results.
- *Prozone effect*: No prozone effect was detected upon 200 mg/dL.
- *Sensitivity*: Δ 7.5 mA.mg/dL.
- *Precision:* The reagent has been tested for 20 days, using two levels of serum in a EP5-based study.

EP5	CV (%)	
	17.85 mg/dl.	35.93 mg/dl.
Total	3.2 %	3.5 %
Within Run	0.8 %	1 %
Between Run	2.4 %	2.4 %
Between Day	2 %	2.3 %

- *Accuracy:* Results obtained using this reagent did not show systematic differences when compared with a reference reagents. Details of the comparison experiments are available on request.

  The results of the performance characteristics depend on the used analyzer.

INTERFERING SUBSTANCES
Bilirrubin (up to 25 mg/dL.) do not interfere. Hemoglobin (≥ 9 g/L), Rheumatoid factors (≥ 200 IU/mL.) and lipemia (≥ 6 g/L), interfere.
Other substances may interfere[5,6].

NOTES
1. Linearity depends on the calibrator concentration.
2. Samples with higher concentrations should be diluted 1/5 in NaCl 9 g/L. and retested again. The linearity limit depends on the sample / reagent ratio, as well the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
3. GPL have instructions for many automatic instruments, available on request.
4. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY

1. Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co., Phipladelphia, 483, 1983.
2. Pesce AJ and Kaplan, LA. Methods in Clinical Chemistry. The CV Mosby Company, St. Louis MO, 1987.
3. Buller HR et al. Critical care Medicine 1982; 10:311
4. Kauffman et al. Am J Med 1978; 65:607.
5. Young DS.Effects of drugs on clinical laboratory tests, 4th ed. AACC Pres, 1995.
6. Friedman and Young. Effects of disease on clinical laboratory tests, 3tn ed. AACC Pres, 1997.

CHEMELEX, S.A.
Pol. Ind. Can Castells. C / Industria 113, Nau J
08420 Canovelles –BARCELONA-
Tel- 34 93 849 17 35 Fax- 34 93 846 78 75

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